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In Vitro and In Vivo Comparison of Argon-Pumped and Diode Lasers for Photodynamic Therapy Using Second-Generation Photosensitizers

Marie J. Hammer-Wilson, MS, Chung-Ho Sun, PhD, Marjan Ghahramanlou, MA, and Michael W. Berns, PhD*

Beckman Laser Institute and Medical Clinic, University of California, Irvine, California

Background and Objective: **Three prototype microchannel-cooled stacked diode array lasers were compared with the currently used conventional argon ion laser-pumped tunable dye lasers for suitability as light sources in photodynamic therapy (PDT) treatment.**
Study Design/Materials and Methods: **The PDT response of Chinese hamster ovary (CHO-K1) cells in culture and SMT-F tumor bearing mice treated with chloro-aluminum sulfonated phthalocyanin (CASPc), benzoporphyrin derivative mono-acid (BPD-MA), and lutetium texaphyrin (Lutex) was determined using each laser light source. Survival of the CHO cells was measured using a cloning assay. Tumor regression/eradication was used to assess response in the mice.**
Results: **Both sources of laser light produced comparable PDT responses in the two systems tested.**
Conclusion: **It would be possible to replace the currently used argon ion laser-pumped dye laser systems with the diode lasers tested. Lasers Surg. Med. 23:274–280, 1998.**

Key words: benzoporphyrin derivative mono-acid; Chinese hamster ovary cells; chloro-aluminum sulfonated-phthalocyanin; lutetium texaphyrin; SMT-F mouse tumor

INTRODUCTION

Photodynamic therapy (PDT) has been shown to be a promising treatment for a variety of neoplasms [1,2]. It is based on the preferential retention of a light reactive compound by a tumor such that the selective absorption of light by the photosensitive compound produces a tumoricidal effect with minimal damage to the surrounding normal tissue [3]. It is important that the delivery of the light be as efficient and reliable as possible. Most frequently the light is delivered by means of a tunable dye laser pumped by an argon ion laser [4,5]. Gold vapour laser [6], copper vapour/dye laser [7], KTP frequency doubled Nd-YAG/dye laser [8], and excimer/dye laser [9] systems also have been used successfully in laboratory trials. Flash lamp-pumped dyed lasers have been found to be ineffective in producing photodynamic action [7,10].

In this study we report the in vitro and in vivo evaluation of three prototype diode lasers for their ability to induce PDT effects at discrete wavelengths (675 nm, 690 nm, and 729 nm), which are matched to the absorption wavelengths of several second-generation photosensitizers.

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*Correspondence to: Dr. Michael W. Berns, Beckman Laser Institute and Medical Clinic, Irvine, CA 92697-1475.

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MATERIALS AND METHODS

Photosensitizers

A stock solution of chloro-aluminum sulfonated phthalocyanin (CASPc) (Ciba-Geigy, Basel, Switzerland), having an average of three sulfonic acid groups per molecule, was prepared at a concentration of 1 mg/ml in PBS and filter sterilized. For the experiments using CHO cells, the final dilution was made using culture medium to 0.1, 0.25, 0.5, and 1.0 μg/ml. For the animal studies, the stock was diluted with PBS to 0.5 mg/ml and each mouse received 5 mg/kg bw.

A stock solution of lutetium texaphyrin (Lutex) (Pharmacyclics, Sunnyvale, CA) at a concentration of 2 mg/ml was prepared in 5% mannitol solution and filter sterilized. For the experiments using CHO cells, dilution to the final concentration of 1.0, 2.0, or 5.0 μg/ml was done using culture medium. For the animal studies, each mouse was injected with stock solution to give 40 mg/kg bw.

The stock solution of benzoporphyrin derivative mono-acid (BPD-MA) (QLT, Vancouver, Canada) was prepared by adding sterile water to the liposomal powder to give a concentration of 1.47 mg/ml. For the experiments using CHO cells dilution to the final concentration of 0.1, 0.25, 0.5, or 1.0 μg/ml was done using culture medium. For the animal studies, the stock solution was diluted with 5% dextrose to give 300 μg/ml and each mouse received 1.5 mg/kg bw.

Laser Systems

For the BPD-MA, 690 nm, and CASPc, 675 nm, experiments a Spectra-Physics model 171 argon laser (Spectra-Physics, Mountain View, CA) with a Spectra-Physics model 375-50 dye laser was used as the conventional light source. For the Lutex experiments 729 nm light was supplied by an argon pumped titanium/sapphire ring laser-(model 899, Coherent, Palo Alto, CA). Verification of the emitting wavelengths was done using a Jobin Yvon model 5/354 UV monochromator (Longjuneau, France).

The diode laser system employed interchangeable laser heads for the specific wavelengths: 675 nm, 690 nm, and 729 nm. (Lawrence Livermore Labs, Livermore, CA). Laser power was controlled by a separate power supply for cw operation (CMS Power Supply, Neptune, NJ). The diode wavelength was thermally selected and maintained at a constant temperature by a refrigerated heat exchanger unit (Neslab, Portsmouth, NH). Both power and cooling utilities were mounted in a mobile utility enclosure, which included quick disconnect fittings to facilitate laser head changes. Additionally, another quick disconnect fitting was provided on the enclosure for supplying external nitrogen through a utility cable to the laser to prevent condensation from forming on the diode emitters. Each laser head consisted of a stack of microchannel-cooled laser diode arrays with built-in SMA connector. Diode array output was conditioned with cylindrical microlenses, which collimated the normally diverging light and allowed focusing using a simple 1.0 cm focal length spherical lens. Each head measured 106 mm × 55 mm × 85 mm and weighed 1.6 Kg. They require a 110 V, 20 A electrical hookup (see Fig. 1).

For all systems, the light beam was directed through a 400 μm fiber (PDT Systems, Santa Barbara, CA) with an uncoated microlens tip. The same fiber was used for all experiments and had an efficiency of 70–80%. Power output for all lasers was determined using a Coherent model 210 power meter (Coherent, Palo Alto, CA) with a thermal disc sensor head, which has a flat spectral response from 0.3–10.6 μm. Power output at the fiber tip was checked at the beginning and end of the cell irradiations, 1.4 W, and after each animal, 80–100 mw depending on the laser.

All lasers were operated in the cw mode. Maximum output from the two argon-pumped systems was ~12 watts from the argon laser portion with 7 watts and 9 watts attainable for the dye and the TiSaph lasers, respectively. The diode lasers all produced 5 watts of cw light. Since the fiber could only withstand 1.5 watts, all systems provided more than sufficient power for the treatments.

Cell Phototoxicity Studies

Chinese hamster ovary cells (CHO-K1, ATCC CCL 61) (American Type Culture Collection, Rockville, MD) were grown in MEM containing nonessential amino acids (Life Technologies/Gibco, Grand Island, NY) supplemented with 0.3 mg L-glutamine/ml and 10% fetal bovine serum (Life Technologies). While the cells were in log growth, they were removed from the flask using 0.25% trypsin, washed, counted using a hemocytometer, and replated in 60 mm culture dishes at a density of 250 cells/dish in fresh medium. After allowing the cells to attach, the dishes were divided into control (photosensitizer only, dye laser light only, diode laser light only) and experimental (photosensitizer + dye laser light; photosensi-

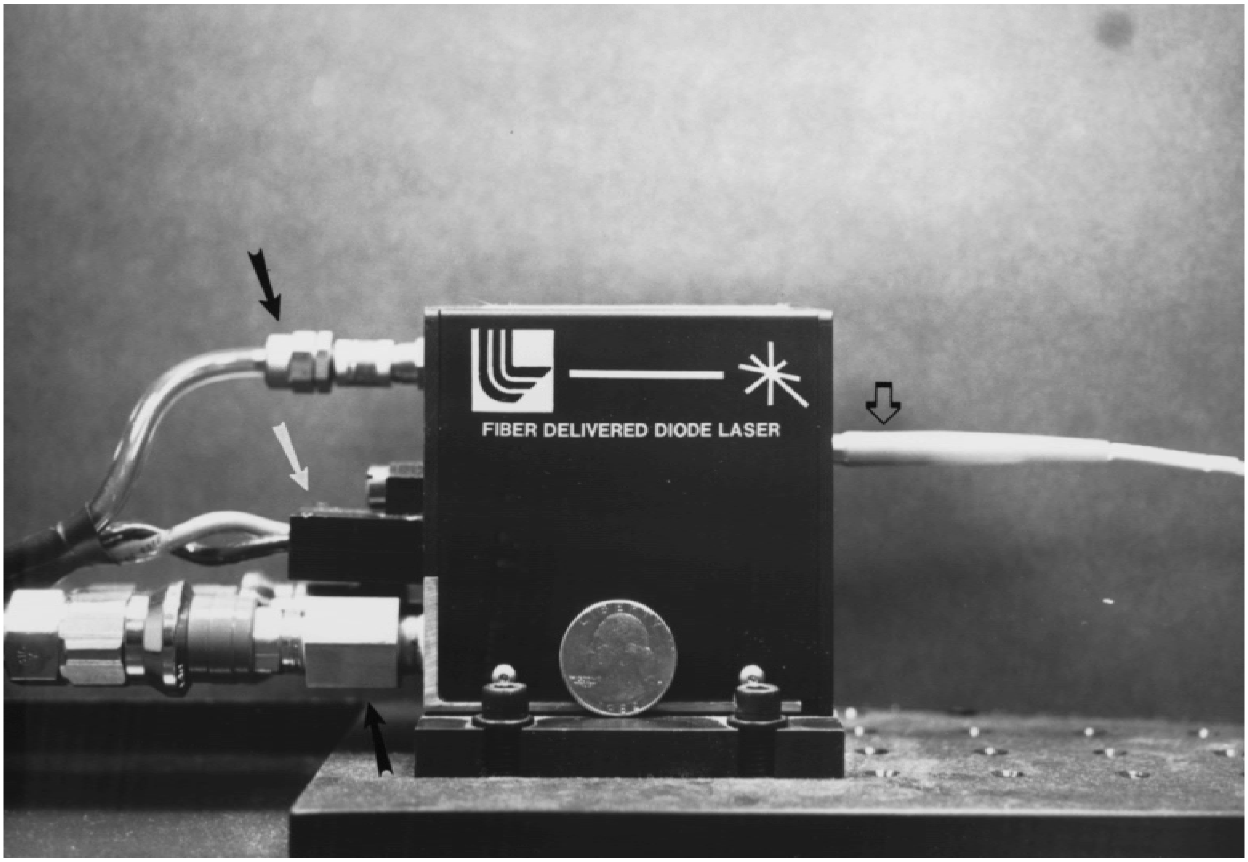

Fig. 1. Side view of diode laser head showing connectors for cooling system (solid black arrows), power supply (solid white arrow) and optical fiber connector (open arrow). For size comparison, the quarter is 2.5 cm in diameter.

tizer + diode laser light) groups. The dishes for PDT or "dark" (photosensitizer only) control were incubated with the desired photosensitizer diluted with medium: 24 hours for CASPc, 1 hour for BPD-MA, and 3 hours for Lutex. The concentration of photosensitizer used ranged from 0.1 μg/ml to 1.0 μg/ml for CASPc, 0.1 μg/ml to 1.0 μg/ml for BPD-MA, and 1.0 μg/ml to 5.0 μg/ml for Lutex. After incubation, the dishes were washed using phosphate-buffered saline (PBS) and fresh medium was added.

Immediately upon addition of the fresh medium to the PDT dishes, each dish was exposed to the laser light for the appropriate time. The CASPc dishes received 10 J/cm^2, the BPD-MA dishes received 5 J/cm^2, and the Lutex dishes received 5 J/cm^2. For all experiments the light was delivered at 28.25 mW/cm^2. At the completion of the light exposure, the dishes were returned to a 5% CO_2 incubator at 37°C for 1 week. Control dishes were washed with PBS and then incubated in fresh medium.

At the end of 1 week, the dishes were washed with PBS, fixed in cold 100% methanol, and then stained with a mixture of 0.5% methylene blue and 0.5% crystal violet in water. Surviving colonies containing a minimum of 150 cells were counted in each dish and the mean for each group of dishes was calculated.

Animal Tumor Model

Female DBA/2 mice (Jackson Labs, Bar Harbor, ME) 6–8 weeks of age, were injected on the flank with a cell suspension of ~5×10^5 SMT-F tumor cells [11] in a volume of 0.05 ml. Cells were prepared by pressing excised tumors from donor mice through a 40 mesh sieve (Sigma, St. Louis, MO), washing the cell suspension in cold, serum-free RPMI 1640 (Life Technologies) with penicillin (100 IU/ml)-streptomycin (100 μg/ml), pelleting the cells at 200 RCF in a laboratory centrifuge, decanting the supernate, and resuspending the cells at a concentration of 10^7 cells/ml in fresh serum-free medium. Where large numbers of animals were to be injected, tumors from multiple

donors were pooled following the sieving of the tumors.

Photodynamic Therapy Treatment of Mice

When the tumors reached 4–7 mm in diameter, the animals were placed into one of the following treatment groups: (1) photosensitizer alone (no light), (2) solvent alone (no light), (3) laser light alone from the conventional laser, (4) laser light alone from the diode laser, (5) photosensitizer and conventional laser light, and (6) photosensitizer and diode laser light.

The photosensitizers BPD-MA and Lutex and their solvents, 5% dextrose (BPD); 5% mannitol (Lutex), were injected via the tail vein. BPD-MA was given 1 hour pre-irradiation and Lutex was given 6 hours pre-irradiation. CASPc and its solvent, PBS, were administered IP 24 hours pre-irradiation. During the laser exposure, the animals were anesthetized with a ketamine (50 mg/kg) and xylazine (10 mg/kg) cocktail given IP to prevent movement of the animal in the beam field. The 675 nm and 729 nm light-exposed tumors were treated with 150 J/cm^2 of cw laser light delivered at a power density of 150 mW/cm^2 for Lutex (729 nm) and 125 mW/cm^2 for CASPc (675 nm). The 690 nm light treated tumors (BPD-MA) received 125 J/cm^2 delivered at 110 mW/cm^2. Each experimental group consisted of ten animals chosen at random from the tumor-bearing population. Each control group contained five mice. Each experiment was done at least twice.

Tumor Size Measurements

Tumor size was monitored for each tumor by measuring two diameters perpendicular to each other using a digital readout caliper (Fowler, Ultracal II, Sylvac, Switzerland). The volume of the tumor was estimated from the formula for a prolate ellipsoid with short axes equal and pi approximated as 3, i.e., $V = 1/2\ L \times W^2$ [12]. Measurements were made at the time of irradiation and three times per week following the treatment until the animal was removed from the study. Animals were removed from the study when their tumors either exceeded 1,700 mm^3 or impaired the animals movement. In order to allow for comparison from experiment to experiment, changes in tumor size were normalized by dividing each tumor's new volume by its starting volume at the time of treatment.

RESULTS

Cell Phototoxicity

Figure 2A shows the survival of CHO-K1 cells following laser irradiation with 10 J/cm^2 of 675 nm light after uptake of CASPc. Survival appears to be dose dependent and there is no "dark" toxicity observed at the highest concentration (1.0 μg/ml) tested. There is no distinguishable difference in response to the two sources of laser light.

Figure 2B shows the survival of CHO-K1 cells following laser irradiation with 5 J/cm^2 of 690 nm laser light after uptake of BPD-MA. Although the photosensitizer appears to act over a very narrow concentration range, no difference is seen in response to the two different sources of laser light.

Figure 2C shows the survival of CHO-K1 cells following laser irradiation with 5 J/cm^2 of 729 nm laser light after uptake of Lutex.

Tumor PDT Response

Figure 3A shows the initial tumor PDT response when the photosensitizer CASPc was used in combination with 150 J/cm^2 of 675 nm laser light. At 10 days post-PDT treatment, the tumor regrowth in the groups treated with the argon/dye laser and the diode laser was found to be indistinguishable ($P = .49$). Long-term survival was found to be better for the argon/dye group, 14 days vs. 12.5 days for the diode group ($P < 0.0001$). Neither group had any tumors that regressed completely.

Figure 3B shows the initial PDT response of tumors treated with BPD-MA and 125 J/cm^2 of 690 nm laser light. At 13 days post-PDT treatment, the tumor size in the two different laser groups was still the same ($P = .54$). After this point the argon/dye irradiated tumors began to regrow more rapidly. A total of 60% of the diode laser mice exhibited complete tumor remission over the observation period of 21 days. Only 20% of the argon/dye irradiated mice had complete tumor remission over the same period.

Figure 3C shows the initial regression of the SMT-F tumors following PDT treatment with 150 J/cm^2 of 729 nm laser light using Lutex as the photosensitizer. Ten days post-PDT treatment, the tumor regrowth in both laser groups was not significantly different ($P = .42$). Only one diode laser irradiated mouse had complete remission during the 25-day observation time. Although the diode laser irradiated mice survived an average of

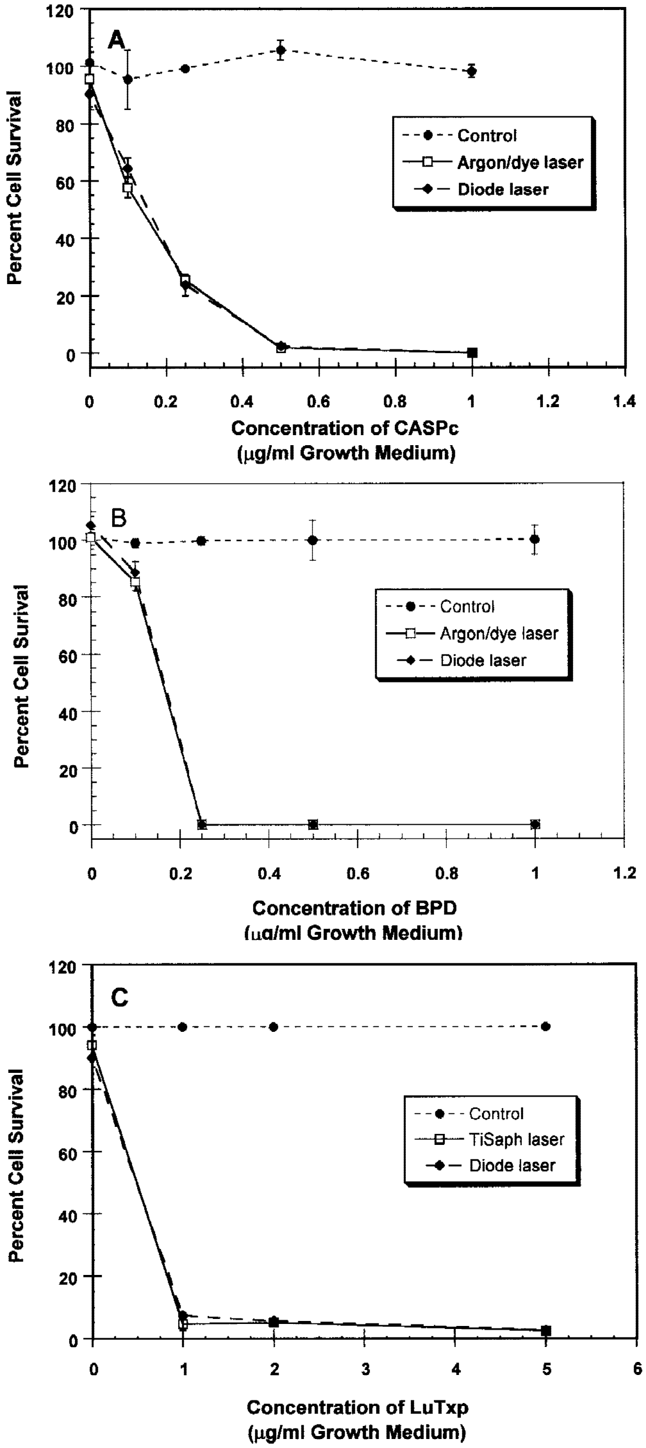

Fig. 2. **A**. Survival of CHO-K1 cells following laser irradiation with 10 J/cm^2 of 675 nm light after 24 hours of uptake of CASPc. **B**. Survival of CHO-K1 cells following laser irradiation with 5 J/cm^2 of 690 nm laser light after 1 hour of uptake of BPD-MA. **C.** Survival of CHO-K1 cells following laser irradiation with 5 J/cm^2 of 729 nm laser light after 3 hours of uptake of Lutex. Each point in A,B,C represents the mean of three dishes. Error bars (A,B,C) = S.E.M.

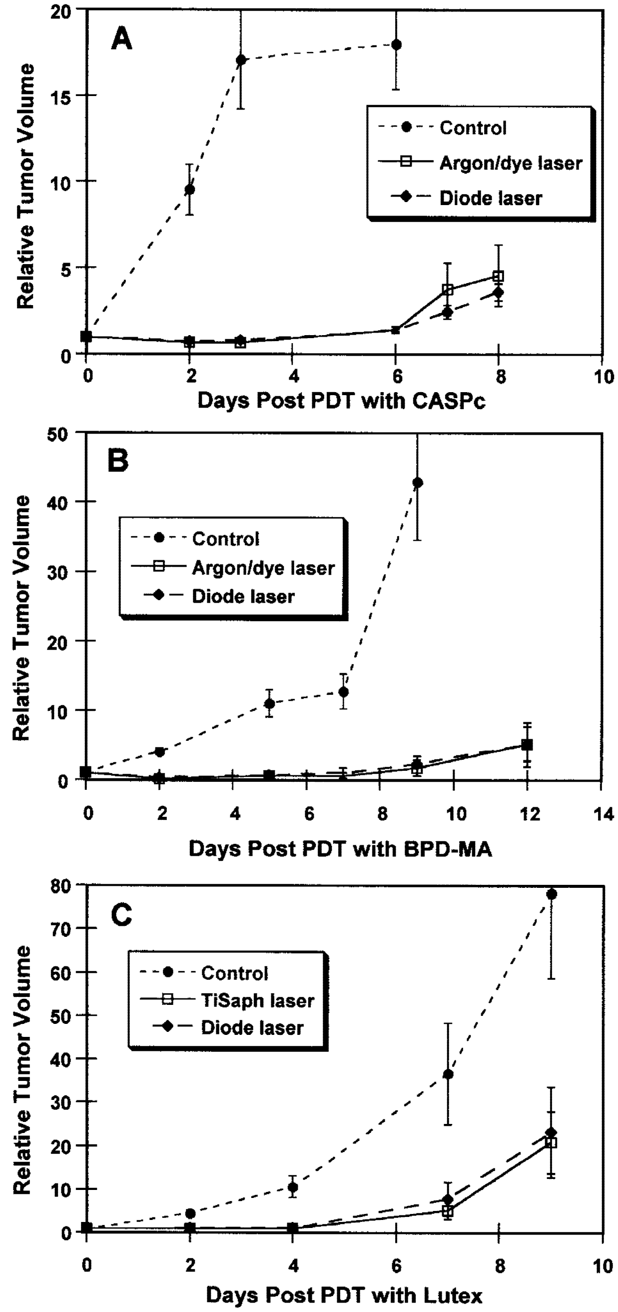

Fig. 3. **A**. PDT response of SMT-F tumors to irradiation with 150 J/cm^2 of 675 nm light from either a diode laser or argon/dye laser source. Mice were treated with 5 mg/kg CASPc 24 hours before irradiation. Control group is a composite of light alone, drug alone and solvent alone mice since they were indistinguishable. **B.** PDT response of SMT-F tumors to irradiation with 125 J/cm^2 of 690 nm light from either a diode laser or argon/dye laser source. Mice were treated with 1.5 mg/kg BPD-MA 1 hour before irradiation. Control group is a composite of light alone, drug alone, and solvent alone mice since they were indistinguishable. **C.** PDT response of SMT-F tumors to irradiation with 150 J/cm^2 of 729 nm light from either a diode laser or argon/dye laser source. Mice were treated with 40 mg/kg Lutex 6 hours before irradiation. Control group is a composite of light alone, drug alone, and solvent alone mice since they were indistinguishable (for A,B,C,P<0.05). Error bars (A,B,C) = S.E.M.

1.5 days longer (15.5 vs. 14.0) than the TiSaph laser irradiated mice, the groups were not statistically different ($P < 0.15$).

DISCUSSION

The argon ion laser-pumped dye lasers currently used for photodynamic therapy have several drawbacks. They are bulky, requiring a large horizontal space to house them. They often require special cooling and electrical hookups. They need maintenance of the dye solutions, both to assure a steady light output and to match the output wavelength with the absorption of the photosensitizer. They contain optics that must be regularly aligned by a skilled technician. Other systems based on dye lasers pumped by other lasers present similar problems.

The three prototype diode lasers we tested eliminate many of these difficulties. They are small and compact. Cooling can be accomplished with compressed nitrogen. There are no dye solutions to change. They do not contain optics that require aligning.

Although we did not have the equipment to measure the light uniformity directly, gross observation of the beam fields produced by the two laser types tested found there to be two visual differences in the light produced by the diode lasers at all three wavelengths tested. First, the edge of the field seemed better defined, i.e., sharper, with the diode laser. Second, the light intensity appeared more uniform with the diode laser giving the light a "flat" rather than the "sparkle" or "shimmer" appearance seen with the pumped lasers. Since the same fiber was used throughout all experiments, it seems unlikely that these differences are due to aberrations introduced by the fiber.

Lifetimes on argon tubes are typically rated as 1,000 hours, although it may be less than that depending on the level of use. Dye lasers generally need their dye changed approximately every 250 hours, but again it is use dependent. We have used each of the diode lasers for ~150 hours with no required maintenance, but have not yet determined their lifespan.

The cost of a complete diode system is comparable to that of the argon portion of a pumped system. Additional diode heads are ~75% the cost of a complete package. Addition of a tunable laser to the pumped system increases the base cost by ~50% (depending on the unit added) and also increases the maintenance (personnel) and supply (dyes, optics, etc.) costs to keep the system operational. The costs cited here for the diode lasers are based on prototype construction costs, whereas those for the argon-pumped systems are based on commercially available systems. Commercial production of the diode systems would undoubtedly bring the price down.

Since the animal experiments took many hours (up to 12 hours in one series), we tested the laser power frequently during the course of the treatments. The traditional argon-pumped systems required periodic adjustment, often showing power drops of greater than 10% over the time required to treat two or three animals (45–60 minutes), as total laser operation time increased. In contrast, the diode lasers were extremely stable and required no adjustment after the initial start-up period of ~30 minutes.

Work by others [13–16] has shown that diode lasers can be effectively used for PDT, even when the laser output is not precisely matched to the absorption of the sensitizer [15,16].

The phototoxicity data on PDT treatment of CHO-K1 cells indicates that there is no difference in killing using light generated by either an argon/pumped dye laser or a diode laser. The short-term volume reduction data for SMT-F tumors would also support the a priori assumption that light from the two laser types is equivalent in producing the PDT response.

However, observation of the PDT-treated tumors for longer periods of time suggests that there may be subtle differences in the light distribution such that the diode-generated light is somehow more efficacious than that produced by the argon/pumped dye system. The greater remission rate seen in the BPD-MA treated tumors would support this possibility. Additional long-term studies will be necessary to further substantiate this observation.

There are two advantages to the systems and photosensitizers we evaluated over those tested previously [13–16]. First, the laser outputs and the photosensitizer absorption peaks are more closely matched than in the other studies. Second, the wavelengths we examined cover a range, 675 nm–729 nm, which goes beyond that of previous studies (652 nm–677 nm), allowing deeper penetration of the light into the tissue. Since we did not detect any disadvantage to the diode systems used in this study, it would be reasonable to pursue development of these systems for clinical use.

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REFERENCES

1. Pass HI. Photodynamic therapy in oncology: Mechanisms and clinical use. J Nat Cancer Inst 1993; 85:443–456.
2. Fisher AMR, Murphree AL, Gomer CJ. Clinical and preclinical photodynamic therapy. Lasers Surg Med 1995; 17:2–31.
3. Henderson BW, Dougherty TJ. How does photodynamic therapy work? Photochem Photobiol 1992; 55:145–157.
4. Doiron DR. Photophysics of and instrumentation for, porphyrin detection and activation. In Doiron DR, Gomer CJ (eds): "Porphyrin Localization and Treatment of Tumours." New York: Alan R. Liss, 1984:41–73.
5. Sam RC, Endriz J. Medical applications of medium to high power diode lasers. SPIE 1994; 2148:260–268.
6. Cowled PA, Grace JR, Forbes IJ. Comparison of the efficacy of pulsed and continuous wave red laser light in the induction of phototoxicity by hematoporphyrin derivative. Photochem Photobiol 1984; 39:115–117.
7. Barr H, Boulos PB, MacRobert AJ, Tralau CJ, Phillips D, Bown SG. Comparisons of lasers for photodynamic therapy with a phthalocyanin photosensitizer. Lasers Med Sci 1989; 4:7–12.
8. Panjehpour M, Overholt BF, DeNovo RC, Petersen MG, Sneed RE. Comparative study between pulsed and continuous wave lasers for photofrin photodynamic therapy. Lasers Surg Med 1993; 13:296–304.
9. Kato H, Kito T, Furuse K, Sakai E, Ito K, Mimura S, Okusima N, Naito K, Suzuki S. Photodynamic therapy in the early treatment of cancer. Gan To Kagaku Ryoho 1990; 17:1833–1838.
10. Bellnier DA, Lin C-W, Parrish JA, Mock PC. Hematoporphyrin derivative and pulsed laser irradiation. In Doiron DR, Gomer CJ (eds): "Porphyrin Localization and the Treatment of Tumours." New York: Alan R. Liss, 1984: 533–540.
11. Pavelic ZP, Porter CW, Allen LM, Mihich E. Cell population kinetics of fast-and slow-growing transplantable tumors derived from spontaneous mammary tumors of the DBA/2 Ha-DD mouse. Cancer Res 1978; 38:1533–1538.
12. Simpson-Herren L, Lloyd HH. Kinetic parameters and growth curves for experimental tumor systems. Cancer Chemotherapy Report 1976; 54:143–174.
13. Katsumi T, Aizawa K, Okunaka T, Kuroiwa Y, Ii Y, Saito K, Konaka C, Kato H. Photodynamic therapy using a diode laser with Mono-L-aspartyl chlorin e6 for implanted fibrosarcoma in mice. Jpn J Cancer Res 1994; 85:1165–1170.
14. DeJode ML, McGilligan JA, Dilkes MG, Cameron I, Grahn MF, Williams NS. An in vivo comparison of the photodynamic action of a new diode laser and a copper vapour dye laser at 652 nm. Lasers Med Sci 1966; 11: 117–121.
15. Lytle AC, Doiron DR, Selman SH. Red diode laser for photodynamic therapy: A small animal efficacy study. SPIE 1994; 2133:178–184.
16. Schmidt MH, Bajic DM, Reichert II KW, Martin TS, Meyer GA, Whelan HT. Light-emitting diodes as a light source for intraoperative photodynamic therapy. Neurosurg 1996; 38:552–557.